# Pathogenicity and Population Dynamics of *Meloidogyne hapla* Associated with *Allium cepa*

A. E. MacGUIDWIN, Department of Plant Pathology, University of Wisconsin, Madison 53706, and G. W. BIRD, D. L. HAYNES, and S. H. GAGE, Department of Entomology, Michigan State University, East Lansing 48823

**ABSTRACT**

MacGuidwin, A. E., Bird, G. W., Haynes, D. L., and Gage, S. H. 1987. Pathogenicity and population dynamics of *Meloidogyne hapla* associated with *Allium cepa*. Plant Disease 71:446-449.

Densities of *Meloidogyne hapla* from 15,000 to 40,000 eggs per plant at seeding retarded the growth of *Allium cepa* 'Krummery Special' under greenhouse conditions. There was a significant ($P = 0.05$) negative linear relationship between the fresh bulb weight of *A. cepa* grown in the field and *M. hapla* density at midseason. *M. hapla* developed and reproduced on *A. cepa* in both greenhouse and field environments. The rate of colonization of *A. cepa* roots and reproduction by *M. hapla* were not affected by increasing nematode inoculum density. According to field census data, there was one generation of *M. hapla* associated with *A. cepa* during the 1981 growing season. The fourfold increase in the *M. hapla* population level was low compared with that reported on other hosts. This seemed to be related to the early senescence of *A. cepa* roots relative to the rate of *M. hapla* development rather than to excessive mortality of *M. hapla* within root systems.

Additional key words: northern root-knot nematode, onion

Seventy-eight species of plant-parasitic nematodes have been recorded from agricultural sites in Michigan (4). Many of these were associated with *Allium cepa* (onion) or other vegetables grown in muck soils. *Pratylenchus*, *Tylenchorhynchus*, and *Meloidogyne* were the genera observed most often during a survey of 5% of Michigan's *A. cepa* acreage in 1980 (6). *Meloidogyne hapla*, the most prevalent root-knot nematode species in Michigan, can be a serious pest of carrot (*Daucus carota* (Vrain)). The importance of *M. hapla* as a pathogen to *A. cepa* has not been adequately assessed, nor has the effectiveness of *A. cepa* in maintaining populations of *M. hapla* been characterized.

*A. cepa* has been reported as a host for *M. hapla*, *M. incognita*, and *M. javanica* (1,7,13). Some reports indicate that *A. cepa* is both intolerant and susceptible to *M. hapla* infection (5,8,10,11), and other sources (1) report only limited yield loss and development of this nematode associated with onion.

The host status of *A. cepa* for *M. hapla* is of interest for several reasons. Information on the tolerance of *A. cepa* to *M. hapla* infection is useful for evaluating control programs for nematodes and soilborne insect pests. Even if *M. hapla* damage to *A. cepa* is minimal, the growth of nematode populations on this host may be sufficient to damage *D. carota*, *Apium graveolens*, or other crops grown in rotation with *A. cepa* in organic soils. The *M. hapla*-onion system is well suited for studies on the within-generation dynamics of phytoparasitic nematodes and can provide important information on the ontogeny of *M. hapla*. Therefore, several experiments were conducted to determine the pathogenicity of *M. hapla* to *A. cepa* and the suitability of *A. cepa* for *M. hapla* population development.

Michigan State Agricultural Experiment Station Journal Article Number 11755. Research conducted as part of a Ph.D. dissertation and supported in part by the Michigan Onion Industry Commission and through NE-133 Regional Research Resources.

Accepted for publication 11 November 1986.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.



## MATERIALS AND METHODS

**Greenhouse pathogenicity and nematode population dynamics experiments.** Ten seeds of *A. cepa* 'Krummery Special' were planted in clay pots containing 800 $cm^3$ (about 868 g dry wt) of pasteurized muck soil with a pH of 6.6 and a soil phosphorus level of 57 ppm. One of the following six densities of *M. hapla* eggs, 0, 100, 1,000, 5,000, 10,000, or 15,000 eggs in 10 ml of water, or water alone, were added to 10 pots of *A. cepa* seeds at planting. The pots were thinned to one seedling at emergence. Plant growth was evaluated at 34,850 accumulative degree hours (base 9 C) after planting. At this time, fresh root, bulb, and leaf weight; dry leaf weight; root and leaf area; and bulb volume were measured. Bulb volume was determined by measuring the volume ($cm^3$) of water displaced when the bulbs were immersed in a water-filled graduated cylinder. One gram of root tissue from each plant was stained in a solution of lactophenol and 0.01% acid fuchsin and examined for nematodes with a stereoscopic microscope. In addition, 100 $cm^3$ of soil from each pot was assayed for nematodes by a modified sugar flotation-centrifugation technique (2), with an extraction efficiency of 35% for *M. hapla* (A. E. MacGuidwin, *unpublished*).

In a similar experiment, six groups of 10 replicate clay pots containing 1,400 $cm^3$ of pasteurized muck soil were infested with 0, 15,000, 20,000, 25,000, 30,000 or 40,000 eggs of *M. hapla*. The inoculation process consisted of placing 1,300 $cm^3$ of soil from each pot in a plastic bag. The nematode inoculum in 10 ml of water was added to the soil in the bag and mixed thoroughly. The inoculated soil was then returned to the pot from which it was removed and covered with 100 $cm^3$ of the uninoculated pasteurized soil. A pregerminated onion seedling, cultivar Krummery Special, was planted in each pot.

A Campbell CR-21 micrologger was used to monitor soil temperature and moisture during the experiment. Six plants from each treatment were removed on each of eight sampling dates, at 2,300 degree hour base 9 C ($DH_9$) intervals. Root length, root weight, and shoot weight of each plant were recorded at each sampling date. The soil and root systems were assayed for *M. hapla* as described previously.

**Field nematode population dynamics experiment.** *A. cepa* 'Krummery Special' seeds were planted on Julian day (JD) 152 (1 June 1981) in 15 three-row beds in a plot at the Michigan State University Muck Research Farm in Bath. Nine consecutive rows of the plot were divided into five 30.77-cm sections and numbered 1–45. About 150 $cm^3$ of soil from a depth of 10–15 cm was removed from each section on JD 152, 181, 195, 210, 224, 237, and 265. A 100-$cm^3$ subsample was assayed for nematodes by a sugar flotation-centrifugation technique (2). The root fragments within the subsample were removed, weighed, stained in a solution of lactophenol with 0.01% acid fuchsin, and examined for nematodes with a stereoscopic microscope. The onions were harvested on JD 265 (22

September 1981) and the fresh bulb weights determined.

The mortality of second-stage juveniles ($J_2$) and of combined third- and fourth-stage juveniles ($J_3$–$J_4$) was estimated using a technique developed by Southwood (12). The counts for each life stage were plotted against time. The area under the resulting stage-frequency curves was integrated to give the total incidence of each stage. Because nematodes required more than 1 day to complete any stage, the total incidence was divided by the time required to complete development of that stage. The values obtained were estimates of the total number of *M. hapla* entering each life stage. The daily survival for each stage was computed by comparing the total number of nematodes that entered each stage (SI = daily survival of stage I): SI = number of individuals in stage I + 1/number of individuals in stage I.

Developmental times at 20 C were estimated for the average soil temperature from the sample site at a 15-cm depth from data presented by Tyler, Vrain et al, and Wong and Mai (13–16). The estimated developmental times were 16 days for $J_2$, 2 days for $J_3$–$J_4$, and 10 days from the last molt until oviposition.

## RESULTS

**Pathogenicity of *M. hapla* on *A. cepa*.** *M. hapla* retarded the growth of *A. cepa* in both of the greenhouse pathogenicity experiments. A significant ($P$ = 0.05) negative linear relationship existed between the relative fresh weight of *A. cepa* (computed as percent weight as compared with control) and inoculum density of *M. hapla*, ranging from 1,000 to 40,000 eggs per plant (Fig. 1). Measurement of selected plants indicated that there was a significant positive relationship between the fresh and dry weight of *A. cepa* 'Krummery Special' ($Y = 0.0633X - 0.0291$, $R^2 = 0.9245$). Data from plants inoculated with 30,000 *M. hapla* eggs were not included in any analysis because of experimental error during the inoculation process.

In both greenhouse pathogenicity experiments, the root fresh weight of *A. cepa* inoculated with 15,000 *M. hapla* eggs was significantly ($P = 0.05$) less than that of uninoculated plants. Differences in the length of root systems in the second greenhouse pathogenicity experiment increased through time for plants inoculated with 15,000 to 40,000 nematode eggs (Fig. 2A–D). There was a similar relationship between fresh root weight and nematode inoculum density.

In the first greenhouse pathogenicity experiment, the mean fresh bulb weight and volume of *A. cepa* inoculated with 15,000 eggs was significantly ($P = 0.05$) less than that of plants grown in noninfested soil but was not different from plants grown in pots inoculated with 100–1,000 eggs per plant according

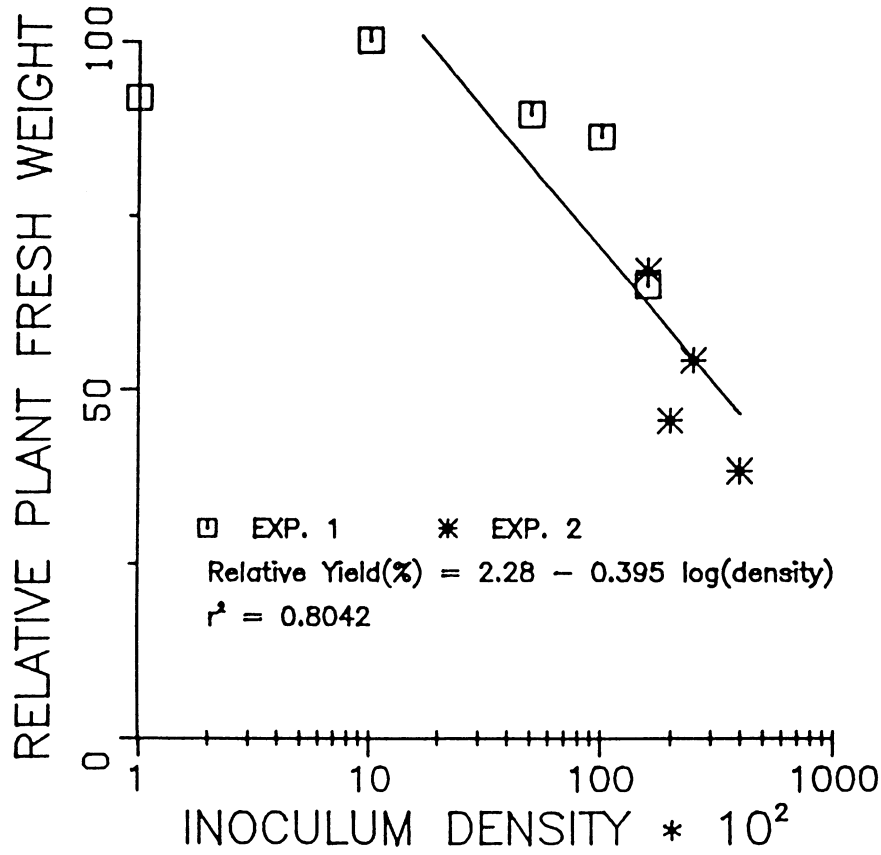


**Fig. 1.** Influence of *Meloidogyne hapla* inoculum density on relative yield (percent yield as compared with control) of *Allium cepa* 'Krummery Special.'

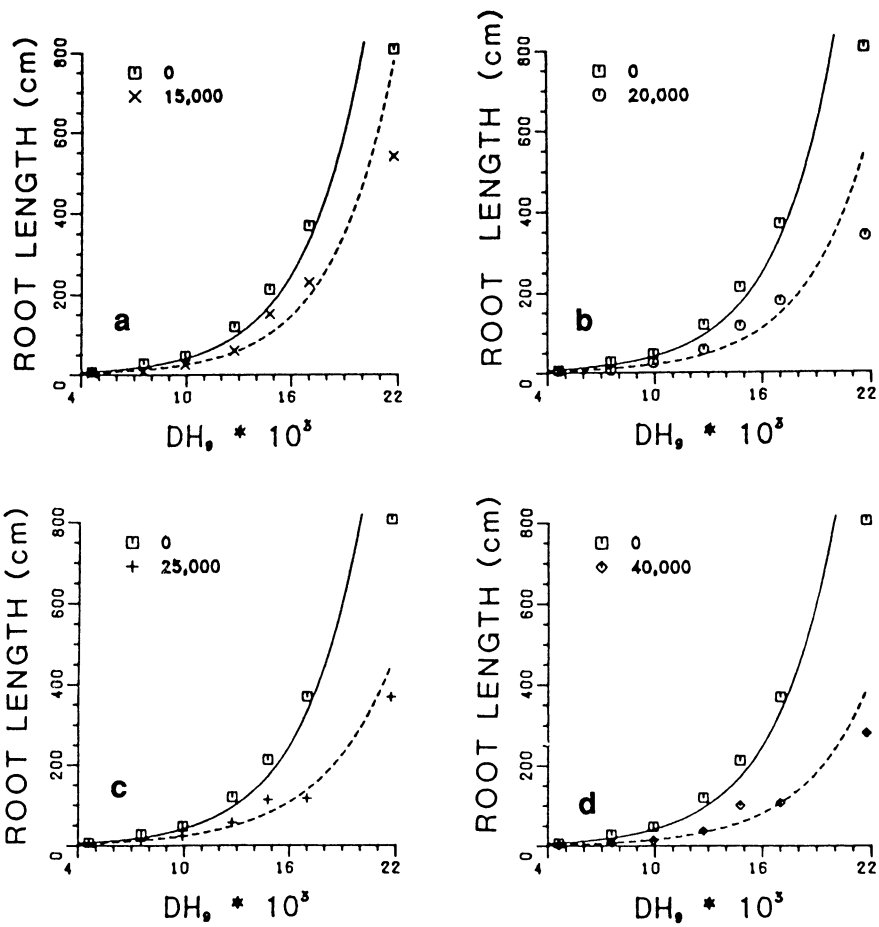


**Fig. 2.** Influence of five initial population densities of *Meloidogyne hapla* on the root length of *Allium cepa* 'Krummery Special' (second greenhouse pathogenicity experiment).

to Duncan's multiple range test. Plants inoculated with 15,000–40,000 *M. hapla* eggs did not form bulbs. Fresh bulb weight measured in the field experiment at harvest was significantly ($P = 0.05$) related to peak *M. hapla* densities occurring in July ($Y = 1{,}195 - 23.24X$) but was not related to initial population densities of *M. hapla* at planting.

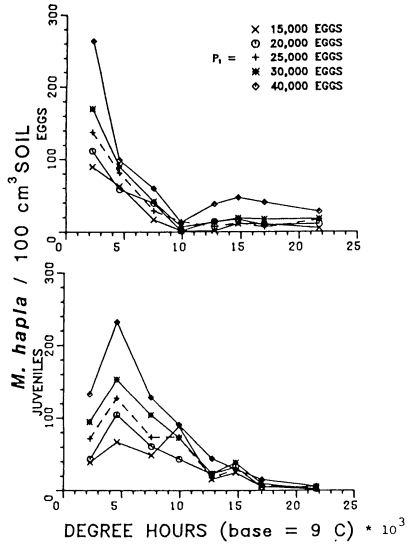


**Fig. 3.** Population dynamics of five initial inoculum levels of *Meloidogyne hapla* eggs and juveniles in *Allium cepa* soil (second greenhouse pathogenicity experiment).

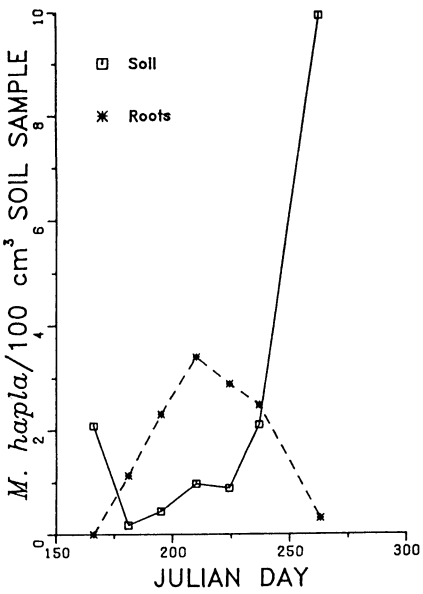


**Fig. 4.** Population dynamics of *Meloidogyne hapla* in *Allium cepa* per 100 cm$^3$ of soil and roots at the Michigan State University Muck Vegetable Research Farm in 1981.

**Population dynamics of *M. hapla* associated with *A. cepa*.** About 20% of the egg inoculum hatched 4,604 $DH_9$ after planting ($Y = 0.006396x - 29.42$; $R^2 = 0.9892$) regardless of inoculum density used in the greenhouse experiments. Population densities of eggs detected in soil samples decreased for all treatments until 12,730 $DH_9$ after planting and inoculation. Levels of $J_2$ in the soil were greatest for all treatments at 4,604 $DH_9$ after inoculation and declined steadily thereafter (Fig. 3).

Final root population densities were similar among treatments for both of the greenhouse experiments. There were no significant differences in the number of nematodes per gram fresh root weight or per root system among plants grown in soil infested with 1,000, 10,000, or 15,000 eggs in the first experiment or among plants from soil inoculated with 15,000–40,000 eggs in the second experiment.

On the final sampling date, the mean numbers of ovipositing females per 0.1 g of root tissue were 1.1, 1.0, 3.2, and 24.4 for plants grown in soil infested with 15,000, 20,000, 25,000, or 40,000 eggs, respectively. Seventy-three days after planting (12,741 $DH_9$), females on plants associated with the lowest inoculum level produced an average of 392 eggs each compared with 329 eggs for females reared on plants receiving the highest inoculum level. Because no significant relationship was detected between the initial nematode egg density and final population levels in root tissue, the data from the eight sampling dates and inoculum levels were pooled. There was no significant relationship between nematode density per plant and egg production when the mean number of eggs per egg sac for an entire root system was regressed on the total number of nematodes observed within that root system.

The number of galled roots was not an accurate reflection of *M. hapla* densities within *A. cepa* roots. On all sampling dates, root gall counts were not significantly correlated with the total number of nematodes or with the number of adult females within a single root system.

In the field experiment, root population densities of *M. hapla* reached a maximum on JD 210 and were very low at harvest on JD 265. *M. hapla* $J_2$ levels in the soil decreased from planting until the first sampling date on JD 181, then increased until the last sampling at harvest (Fig. 4). Comparing the final soil plus root population density ($P_f$) of *M. hapla* with initial soil densities ($P_i$) indicated a seasonal rate of increase of 4.9.

All root-inhabiting stages of *M. hapla* were present from JD 210 through the end of the experiment (Table 1). Population densities of $J_2$ peaked at the second sampling date on JD 195. $J_3$–$J_4$ and preovipositing adults of *M. hapla* reached maximum densities on JD 210. The occurrence of ovipositing females was first noted on this sampling date and was greatest on JD 224, or 73 days after planting. The survival rates for the $J_2$ and $J_3$–$J_4$ were 0.7944 and 1.0763, respectively.

## DISCUSSION

The total plant weight of *A. cepa* 'Krummery Special' was reduced up to 60% by a $P_i$ of 40,000 *M. hapla* eggs per plant. This is equivalent to 8,000 juveniles per plant, based on a 20% rate of egg hatch. The data were better described by linear regression of the transformed values than by Seinhorst's model for nematode damage (9). Olthof and Potter (8) reported that the commercial yield of Copper Gem onions was reduced 64% by a $P_i$ of 54,000 $J_2$ per plant and that a $P_i$ of 6,000 $J_2$ per plant was sufficient to cause economic damage. The reduction of shoot and bulb fresh weight observed under greenhouse and field conditions, respectively, support Olthof and Potter's findings. A survey of onion acreage in Michigan (6) revealed that soil densities of *M. hapla* at planting are generally lower than the levels used in either the Olthof and Potter or current study. The possibility of *A. cepa* yield loss caused by *M. hapla*, however, should not be discounted, because the ability to accurately measure nematode density in the field is limited by errors in sampling and assay procedures.

*M. hapla* developed and reproduced on *A. cepa* 'Krummery Special' in both the greenhouse and field experiments. Although the fresh weight of *A. cepa* grown in the field was not related to the initial soil levels of a natural *M. hapla* infestation, there was a significant relationship between nematode density at midseason and *A. cepa* yield. The increase of *M. hapla* population densities on *A. cepa* is relatively low compared with that on *D. carota* (6). The results of this and several earlier studies (5,10,12), however, show that *M. hapla* can be maintained on *A. cepa* in sufficient numbers to be damaging to subsequent crops of *D. carota* or other vegetables. Most *M. hapla* that established within *A. cepa* roots survived. In fact, the survival

**Table 1.** Incidence of life cycle stages of *Meloidogyne hapla* recovered from 0.1 g of onion root tissue grown in 1981 in organic soil under commercial onion production conditions

| Julian date | Second-stage juveniles | Third- and fourth-stage juveniles | Preovipositing females | Ovipositing females |
|---|---|---|---|---|
| 181 | 1.13 ± 0.27[a] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 195 | 2.24 ± 0.41 | 0.04 ± 0.03 | 0.02 ± 0.02 | 0.00 ± 0.00 |
| 210 | 1.29 ± 0.22 | 0.04 ± 0.12 | 1.58 ± 0.33 | 0.13 ± 0.05 |
| 224 | 0.51 ± 0.12 | 0.18 ± 0.07 | 1.20 ± 0.24 | 0.93 ± 0.24 |
| 237 | 0.96 ± 0.30 | 0.07 ± 0.04 | 0.76 ± 0.18 | 0.69 ± 0.16 |

[a] Mean ± standard error.

of $J_2$ may have been underestimated if most mortality occurs at the onset of the stage, because Southwood's method (12) assumes that mortality is distributed evenly throughout the life stage.

Although it was not possible to measure the total egg production of female nematodes, the reproduction of *M. hapla* did not seem to be impaired on *A. cepa* in the greenhouse experiment. It appears, then, that the major factor limiting the increase of *M. hapla* associated with *A. cepa* in the field is the phenology of the host plant. According to our census data, only one generation of *M. hapla* occurred in 1981, and most females had not commenced oviposition before the senescence of *A. cepa* roots.

The relationship between the initial inoculum level of *M. hapla* and final population level was difficult to characterize, primarily because of the continuous *A. cepa* root growth and movement of nematodes into root tissue. Because the period of survival of juveniles in the soil is less than the time required for the development and eclosion of eggs, this problem could have been avoided if $J_2$ rather than eggs had been used as inoculum.

Generally, it seemed that egg hatch and *M. hapla* levels within roots were independent of nematode population density. It is possible that the number of *M. hapla* that entered *A. cepa* roots was related to the inoculum density but that the survival of nematodes within roots was reduced when population levels were high. We anticipated testing this possibility but could not measure stage-specific survival in the greenhouse test because of overlapping incidence of life stages. Counts of eggs and empty egg shells within egg masses provided an estimate of the total number of eggs produced and indicated that there was no relation between the density of nematodes per root system and the reproduction of *M. hapla* females. The results of this research were also used to develop a computer model simulating the population dynamics of *M. hapla* associated with *A. cepa* in Michigan (6).

As previously reported (3), root galls were a poor indicator of *M. hapla* population levels within roots. Gall indices, when used as a measure of *Meloidogyne* population development, should be supported by other measures of nematode abundance.

**ACKNOWLEDGMENT**

We wish to thank John Davenport for his technical assistance with the research.

**LITERATURE CITED**

1. Chitwood, B. G. 1951. Root-knot nematodes II. Quantitative relationships of the root-knot nematode *Meloidogyne hapla* Chitwood, 1949, with tomatoes, onions, and lima beans. Plant Soil 3:47-50.
2. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
3. Kinloch, R. A., and Allen, M. W. 1972. Interaction of *Meloidogyne hapla* and *M. javanica* infecting tomato. J. Nematol. 4:7-16.
4. Knobloch, N., and Bird, G. W. 1981. Plant-parasitic nematodes of Michigan: with special reference to the genera of the Tylenchorhynchinae (Nematoda). Res. Rep. 419, Mich. State Univ. Agric. Exp. Stn. 35 pp.
5. Kotcon, J., Bird, G. W., Rose, L. M., and Dimoff, K. 1985. Influence of *Glomus fasciculatum* and *Meloidogyne hapla* on *Allium cepa* in organic soils. J. Nematol. 17:55-60.
6. MacGuidwin, A. E. 1983. Pathogenicity and ecology of *Meloidogyne hapla* associated with *Allium cepa*. Ph.D. dissertation. Department of Entomology, Michigan State University, East Lansing. 237 pp.
7. Martin, G. C. 1961. Plant species attacked by root-knot nematodes (*Meloidogyne* spp.) in the Federation of Rhodesia and Nyasaland. Nematologica 6:130-134.
8. Olthof, T. H. A., and Potter, J. W. 1972. Relationship between population densities of *Meloidogyne hapla* and crop losses in summer-maturing vegetables in Ontario. Phytopathology 62:981-986.
9. Seinhorst, J. W. 1965. The relation between nematode density and damage to plants. Nematologica 11:137-154.
10. Sherf, A. F., and Stone, L. W. 1956. Field control of root-knot nematode in onion muck by the use of fumigants. (Abstr.) Phytopathology 46:242.
11. Smith, J. J. 1964. Host-parasite relationships of *Allium cepa* L. and *Meloidogyne hapla* Chitwood, 1949. M.S. thesis. Cornell University, Ithaca, NY. 44 pp.
12. Southwood, T. R. E. 1966. Ecological Methods. Methuen & Co., London. 279 pp.
13. Tyler, J. 1933. Development of the root-knot nematode as affected by temperature. Hilgardia 7:391-415.
14. Vrain, T. C. 1982. Relationship between *Meloidogyne hapla* density and damage to carrots in organic soils. J. Nematol. 14:50-57.
15. Vrain, T. C., Barker, K. R., and Holtzman, G. I. 1978. Influence of low temperature on rate of development of *Meloidogyne incognita* and *M. hapla* larvae. J. Nematol. 10:166-177.
16. Wong, T. K., and Mai, W. F. 1973. Effect of temperature on growth, development and reproduction of *Meloidogyne hapla* in lettuce. J. Nematol. 5:139-141.